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REMARKS

Claims 1-38, 41-43, 72, 73, and 75-86 are active in this application. Support for the amendment to Claim 1 is found in Claims 13, 14, and 15 as well as the specification on page 7, lines 16-17. Support for Claims 76-86 is found in the specification on page 1, lines 17-19. The specification at page 30 has been amended to correct a typographical error. No new matter is added by these amendments.

Applicants wish to thank the Examiner for withdrawing the restriction requirement and examining Claims 1-75. In light of the amendments and remarks submitted herein, Applicants respectfully request favorable reconsideration and allowance of all pending claims.

The rejection of Claims 1-19, 21-56, 58-75 under 35 U.S.C. § 103(a) over 35 U.S.C. § 103(a) over Yoshikawa et al. is not tenable for the following reasons.

The method described in Yoshikawa et al. is the opposite of what is presently claimed (see MPEP § 2141.02: “PRIOR ART MUST BE CONSIDERED IN ITS ENTIRETY, INCLUDING DISCLOSURES THAT TEACH AWAY FROM THE CLAIMS”). In particular, Applicants draw the Examiner’s attention to the process in Claim 1 which comprises **(a)** incubating a denatured transglutaminase in an acidic aqueous medium, **(b)** diluting the denatured transglutaminase in the acidic aqueous medium; and **(c)** adjusting the pH of the aqueous medium to a neutral pH by the addition of an alkali. In contrast, the method described in Yoshikawa et al. solubilized an insoluble fused protein with a protein denaturant by increasing the pH to an alkaline pH (contrast to (a) in the present method) and subsequently lowering the pH (contrast to (c) in the present method).

There is no description to modify the teachings of Yoshikawa et al. to replace the solubilization of the insoluble protein at an alkaline pH with the acidic pH as in Claim 1. Furthermore, there is no description to replace the subsequent lowering of the pH in

Yoshikawa et al with the adjustment to a neutral pH by adding an alkali as in Claim 1. One would simply not modify the teachings of Yoshikawa et al because doing so would change the “principle of operation” of the Yoshikawa et al method (see MPEP § 2143.01).

Therefore, withdrawal of this ground of rejection is requested.

To the rejection of Claims 1-10, 13-19, 21-47, 50-56 and 58-74 over Ejima et al., this rejection is also not tenable for the following reasons.

Ejima et al. describe the purification of human interleukin-6 by solubilizing an inclusion body under denaturing conditions and then immediately removing the denaturant without any dilution (see column 1 on page 303 of Ejima et al.: “the native conformation of hIL-6 was obtained by rapidly removing the denaturant from the oxidized hIL-6 solution without any dilution.”). Since the present claims include diluting the denatured transglutaminase by about 5-fold to about 400-fold and the present application describes that the process of producing transglutaminase involves this dilution is important for the recovery of the active transglutaminase (referring to the discussion on pages 10-12 of this application), Ejima et al. fail to describe every limitation of the present claims as is required to establish a rejection under 35 U.S.C. § 103. Therefore, withdrawal of this ground of rejection is requested.

Concerning the rejection of Claims 1-75 under 35 U.S.C. § 112, first paragraph, this rejection is respectfully traversed for the following reasons.

The specification describes and demonstrates that an active transglutaminase can be recovered by first incubating the denatured enzyme in an acidic aqueous medium, diluting the denatured enzyme, and adjusting the pH to a neutral pH by adding an alkali to the medium. This is described in the specification on pages 10-12 and therefore the claims should not be limited to the embodiments specifically set forth in the Examples. As a result withdrawal of this ground of rejection is respectfully requested.

The rejection of Claims 1, 9, 23-34, 36, 38, and 44-46 under 35 U.S.C. § 112, second paragraph have been addressed, in part by amendment and is respectfully traversed, in part, for the following reasons.

With respect to the terms acidic pH and neutral pH these terms have a well defined and known meaning in the field. The specification on page 10, lines 23-26 describe acidic pH and likewise on page 12, lines 14-19 neutral pH is described. These definitions are entirely consistent with the definitions commonly known and used as illustrated in the attached pages 435-436 of the textbook entitled *Chemistry*, 2nd edition (Bailar et al (ed.), Academic Press, Orlando, FL (1984)).

All other remaining grounds of rejection have been addressed by appropriate amendment and therefore withdrawal of this ground of rejection is requested.

Lastly, to the rejection of Claim 75 under 35 U.S.C. § 112, first paragraph, Applicants respectfully point out that this claim is supported by the specification on page 32, Example 16, which states “After dilution the enzyme exhibited mobility clearly different from that in the native state, showing that the structural state formed by the dilution is different from the native state.” This is further supported by the results shown in Figure 13, the figure legend of which is provided on page 6. In this Figure, lane 2 corresponds to the denatured transglutaminase after dilution whereas lanes 1, 3 and 4 correspond to enzymes in the native state. Thus, it is clear that the enzyme mobility in lane 2 is retarded relative to the native enzymes as shown by its higher position on the gel, which in turn demonstrates that this enzyme has a lower mobility than that of the native enzyme.

Therefore, this claim is, in fact, adequately described according to 35 U.S.C. § 112, first paragraph and as such withdrawal of this ground of rejection is respectfully requested.

In light of the foregoing, Applicants submit that the present application is now ready for allowance. Early notification of such allowance is kindly requested.

Respectfully submitted,

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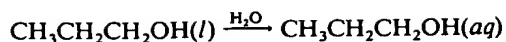
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Exercise Write chemical equations describing the dissolution of the following substances in water: (a) gaseous oxygen, O_2 ; (b) solid potassium bromide, KBr ; (c) solid sodium hydroxide, NaOH ; and (d) solid silver chloride, AgCl . *Answer* (a) $\text{O}_2(g) \xrightarrow{\text{H}_2\text{O}} \text{O}_2(aq)$, (b) $\text{KBr}(s) \xrightarrow{\text{H}_2\text{O}} \text{K}^+ + \text{Br}^-$, (c) $\text{NaOH}(s) \xrightarrow{\text{H}_2\text{O}} \text{Na}^+ + \text{OH}^-$, (d) $\text{AgCl}(s) \xrightarrow{\text{H}_2\text{O}} \text{Ag}^+ + \text{Cl}^-$

14.8 ACIDS AND BASES; H^+ AND OH^-

Acids and bases were first recognized as specific classes of compounds because of the distinctive properties exhibited by their aqueous solutions. The classic acid and base properties and a few common acidic and basic substances are listed in Table 14.6.

In the 1880s Svante Arrhenius, a Swedish chemist, developed a theory for ionization in aqueous solutions. Part of his theory explains that the classic acid properties are imparted to an aqueous solution by the hydrogen ion, H^+ , and the basic properties by the hydroxide ion, OH^- . An acidic aqueous solution contains a greater concentration of H^+ ions than of OH^- ions. A basic aqueous solution, better called an alkaline aqueous solution, contains a greater concentration of OH^- ions than H^+ ions. (The term "alkaline" is preferable because "basic" also has other

Table 14.6

Acids and Bases Early chemists blithely tasted their chemicals. Experience has shown that this can be a fatal mistake. *Never* taste any laboratory chemicals. The acidity of vinegar is due to acetic acid (CH_3COOH). Fruits and vegetables derive their acidity from various organic acids. Gastric juice contains hydrochloric acid (HCl). Carbonated beverages are acidic because of the reaction of CO_2 with water. Aspirin and vitamin C are organic acids, acetylsalicylic acid and ascorbic acid, respectively. Household ammonia is alkaline because of the reaction of ammonia gas (NH_3) with water, and milk of magnesia contains magnesium hydroxide [$\text{Mg}(\text{OH})_2$]. Soap and detergents contain various organic and inorganic bases.

Acids	Bases
Sour taste	Bitter taste
Change colors of indicators, e.g., litmus turns from blue to red; phenolphthalein turns from red to colorless	Slippery feeling
React with active metals to give hydrogen, e.g., $\text{Zn}(s) + 2\text{H}^+ \longrightarrow \text{Zn}^{2+} + \text{H}_2(g)$	Change colors of indicators, e.g., litmus turns from red to blue; phenolphthalein turns from colorless to red
Acidic properties disappear in reaction with a base	Basic properties disappear in reaction with an acid
<i>Some Acidic Substances</i>	<i>Some Alkaline Substances</i>
Vinegar	Household ammonia
Tomatoes	Baking soda
Citrus fruits	Soap
Carbonated beverages	Detergents
Black coffee	Milk of magnesia
Gastric fluid	Oven cleaners
Vitamin C	Lye
Aspirin	Drano
Saniflush	

Table 14.7
Common Acids and Bases Nitrous acid is known only in solution.

Strong acids	
HCl	Hydrochloric acid
HBr	Hydrobromic acid
HI	Hydroiodic acid
HNO ₃	Nitric acid
H ₂ SO ₄	Sulfuric acid
HClO ₄	Perchloric acid
Weak acids	
CH ₃ COOH	Acetic acid
HF	Hydrofluoric acid
H ₃ PO ₄	Phosphoric acid
HNO ₂	Nitrous acid
Strong bases	
NaOH	Sodium hydroxide
KOH	Potassium hydroxide
Weak base	
NH ₃	Ammonia

Water-ion definitions:

Acid: H compound $\xrightarrow{\text{H}_2\text{O}} \text{H}^+$

Base: OH compound $\xrightarrow{\text{H}_2\text{O}} \text{OH}^-$

meanings. "Alkaline" derives from "alkali," an old name for substances with the classic properties of bases.)

The Arrhenius definitions of acids and bases, derived as they are from the presence of hydrogen and hydroxide ions in aqueous solutions, can be called the *water-ion* definitions. An acid (*water-ion*) is a substance that contains hydrogen and yields hydrogen ions in aqueous solution. The common *strong acids* are all water-ion acids. They are molecular compounds that are strong electrolytes and are 100% ionized in aqueous solution. Some are binary acids like hydrochloric acid (Equation 14.10) and others are oxo acids, such as nitric acid:

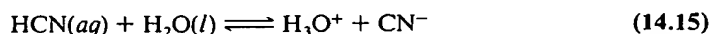


A *base (water-ion)* is a compound that contains hydroxide ions and when it dissolves in water dissociates to give hydroxide ions. The bases defined in this way are all ionic compounds and strong electrolytes. Those hydroxides that are soluble or moderately soluble (the alkali metal hydroxides, and calcium, strontium, and barium hydroxide) give high concentrations of hydroxide ions in solution and are referred to as *strong bases*. The most common strong bases are sodium and potassium hydroxide:



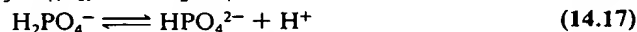
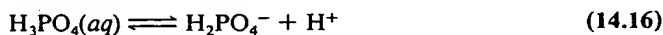
The majority of metal hydroxides, although they are all strong electrolytes, are only slightly soluble (see Table 6.3), and therefore give lower concentrations of OH⁻ in aqueous solution.

Many hydrogen-containing molecular compounds are weak electrolytes and therefore *weak acids*, for they are less than 100% ionized. For example, acetic acid (Equation 14.11) and hydrogen cyanide, known in its aqueous solution as hydrocyanic acid, are both weak acids and weak electrolytes.

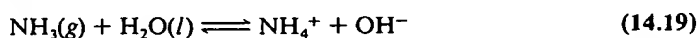


You should become familiar with the names and formulas of the common acids and bases listed in Table 14.7.

Sulfuric acid and phosphoric acid (see Table 14.7), and other acids like them that contain more than one ionizable hydrogen atom, are known as **polyprotic acids**. Such acids ionize in steps, for example, for phosphoric acid:



Broader definitions of acids and bases than those of Arrhenius have been introduced over the years as it became desirable to categorize more types of substances as acids and bases. To give a simple example of a substance that is clearly a base although not an ionic hydroxide, consider ammonia, which yields hydroxide ions in solution according to the following equilibrium:



Ammonia is the most common weak base. (A useful way of defining acids and bases that includes ammonia as a base is discussed at length in Chapter 20.)

EXAMPLE 14.5 Acids and Bases

Write equations for the interaction of water with (a) perchloric acid; (b) nitrous acid (aq); and (c) the weak base aniline, C₆H₅NH₂.

(a) Perchloric acid, a strong acid, is completely ionized.

